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AN INVESTIGATION CONCERNING THE POTENTIAL FOR USE OF POLYLACTIC/POLYGLYCOLIC ACID CONFLUENT SHEETS IN THE TREATMENT OF OSSEOUS DEFECTS

BY

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SIGNIFICANCE

Polylactic/polyglycolic acid combinations (alpha polyamides) are biodegradable materials commonly used in suture materials and surgical meshes for the temporary support of abdominal organs. The materials are well known in the clinical literature and have been in common usage without risk for over two decades (1-5). The polyamides arae rich in polyester linkages that are degraded in aqueous envirnonments by hydrolysis (5-7). Various investigators have shown that the pure form of polyglycolic acid (PGA) degrades over a period of 5 months whereas pure polylactic acid (PLA) takes about 6.5 months (7, 8). Copolymers of PLA/PGA have been synthesized that degrade within a few weeks to several months depending upon the ratios employed and the specific sequencing of the chemical units (7, 9). Further, the alpha polyamides show no adverse host tissue reactions when implanted in numerous animal models (10-15). As the materials are biodegradable, the rate of which can be controlled by changing the material density, we are suggesting that it may be employed as a matrix for osseous grafting, for the occlusion of large bony defects, for soft tissue contour defects, and also as a bone plating system. All of these applications are based upon the assumption that normal host fibrous and/or osseous tissues will replace the PLA/PGA materials as they undergoe biologic degradation. The potential of PLA/PGA combinations for use in osseous grafting and bone stabilization systems as not been extensively investigated. Indeed, if our assumptions prove correct, PLA/PGA combinations could accomplish all the functions of present day

surgical titanium but with the distinct advantage of not requiring a secondary surgery for removal of fixation plates, etc.

Thus, the purpose of this study is to determine the fate of a specific PLA/PGA combination when used as a continuous sheet within osseous defects or when placed on either side of defects in the manner of a bone plate. Further, the type of tissue replacement is to be determined as well as the percent fill of the defect with new bone.

MATERIALS AND METHODS

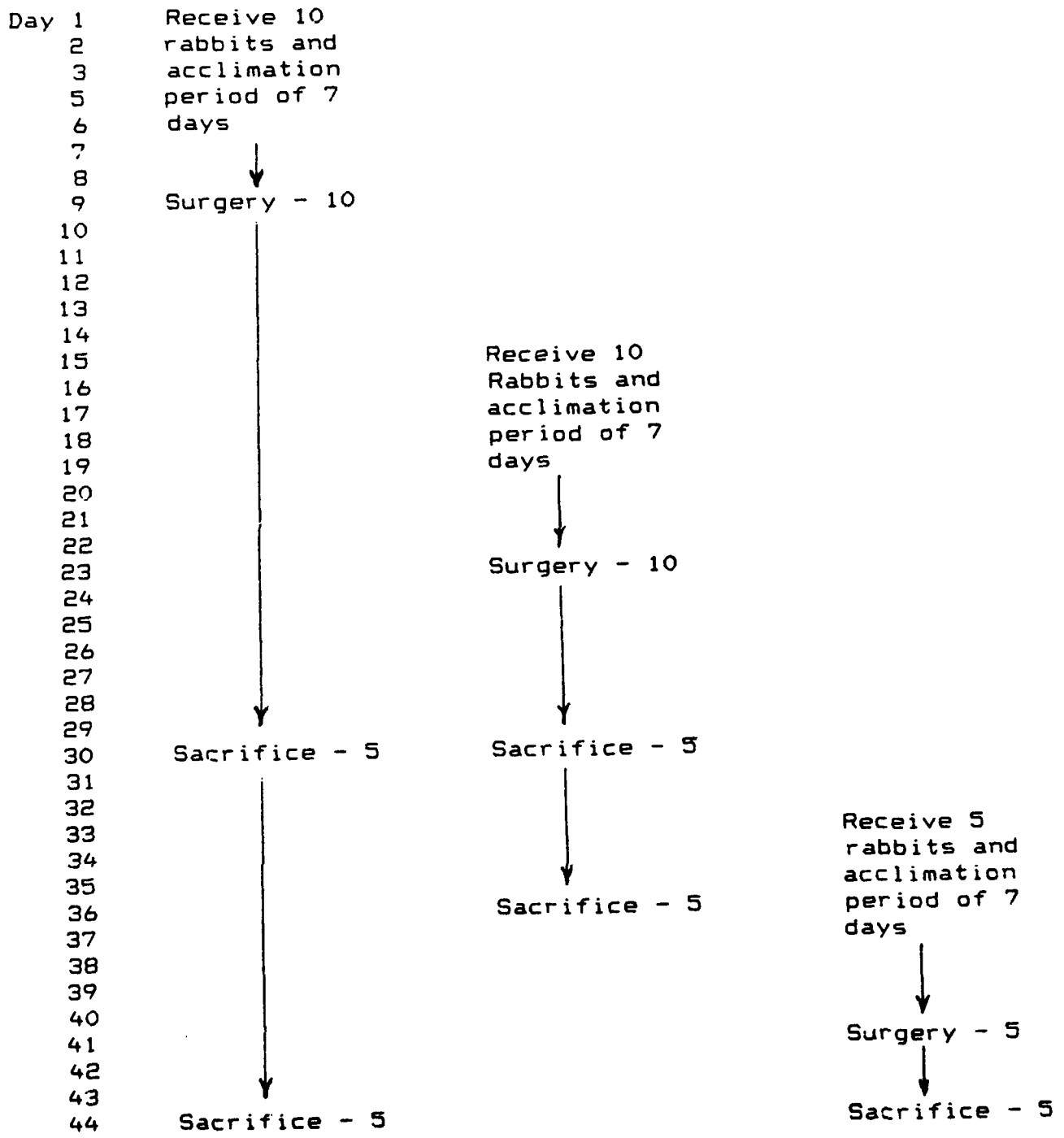
Animal Model: Twenty-five adult male New Zealand albino rabbits between the ages of 9 and 12 months and weighing approximately 3000 gm were obtained from a licensed commercial vendor through the Laboratory Animal Care (LAC) facility of the University of Missouri-Kansas City. Animals were caged separately in a standard manner and fed Purina Rabbit Chow plus water ad libitum. All animals were allowed one week of acclimation to their new environment before initiating the experimental protocol.

Animal Housing and Surgical Schedule: Rabbits were housed, in the LAC facility, located on the third floor of the UMKC, School of Medicine, 2411 Holmes, Kansas City, MO. All surgical procedures, post-surgery care, and animal sacrifice were performed in the same facility.

Animals were ordered in groups of ten, to be delivered at staggered intervals of approximately fourteen days. The last order for animals requested six rabbits as one animal was lost during the post-surgery recovery period. Thus, the day-by-day

day-by-day schedule appeared as follows:

Housing, Surgery and Sacrifice Schedule



Implementation of the the above schedule, determined a maximum daily census for animal housing to be twenty rabbits for fifteen days (days 16-30 of schedule).

Anesthesia: Upon completion of the acclimation period, animals were prepared for the surgical implantation of the PLA/PGA confluent sheets. Food and water were withheld for a twelve hour period prior to surgery. Anesthesia was obtained using the following regimen:

(1) Preanesthetic intramuscular (IM) administration of Atropine using a dosage of 0.06-0.08 mg/kg.

(2) Anesthetic induction using IM administration of Xylazine 5 mg/kg plus Ketamine hydrochloride 30-45 mg/kg.

(3) Anesthetic maintenance was accomplished by using IM Ketamine as required.

Anesthesia was confirmed by loss of reflex when pinched on the abdomen and/or loss of eye-blinking reflex. Subsequent to anesthesia, cranial hair was shaved and removed by vacuum. The resulting surgical field was then swabbed with betadine surgical scrub solution.

Surgical and Implantation Procedures: Two full-thickness semilunar flaps were raised using sharp incision and blunt dissection. The anterior border of the ears formed the base of each flap. Using a surgical steel trephine dental-implant bur in a slow-speed high-torque dental handpiece, three osseous defects, approximately 4 mm in diameter, were created in the calvaria equidistant from the mid-sagital suture and from each other. Defects penetrated the complete thickness of calvarial bone but did not violate the integrity of the dura mater. A new sterile

bur was used for each animal. Further, during osseous penetration, the field was continuously irrigated with sterile saline containing penicillin (100 units/ ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), and Fungizone (2.5 μg/ml).

Complete removal of all residual osseous fragments was insured by irrigation and simultaneous suctioning. This step was particularly important as osseous grindings left in defects may act as autogenous grafts and bias the experimental evaluation.

One cranial defect in each animal was assigned by random selection and packed with the PLA/PGA implant material. A second defect, again selected by random assignment, was treated by placement of sheets of the implant material on either side of the osseous cavity, leaving the defect unfilled. This latter proceddure required the implant material to be placed in direct contact with the osseous surface, thereby being interposed between bone and dura mater and/or bone and scalp connective tissues. The remaining defect was left untreated and serve as the control. Scalp flaps were repositioned and held in place with polyglycolic 4-0 sutures. The scalp overlying implant and control sites was tattooed to aid identification of surgical areas for biopsy at the time of sacrifice.

Post-Surgical Care: All post-surgical care was the responsibility of the investigating team, i.e., Department of Oral and Maxillofacial Surgery, Truman Medical Center. As it was reasonable to expect some post-surgical pain or distress, a regimen of 1-5 mg/kg of Diazepam administered IM was used on an "as needed basis". All animals were checked daily for the first 3-4 days

post-surgery for changes in appetite, physical activity and general appearance.

Sample Procurement and Analysis: Animals were randomly selected for sacrifice using Pentobarbital, 75-100 mg/kg by IV administration until cessation of respiratory and cardiac function was apparent. The schedule of sacrifice was be as follows:

5 animals - day three post-surgery
5 animals - day seven post-surgery
5 animals - day fourteen post-surgery
5 animals - day twenty-one post-surgery
5 animals - day thirty-five post-surgery

At the specified time interval, the implant and control surgical sites were biopsied and tissue placed in labeled bottle containing 10% buffered formalin. After fixation, specimens are demineralized in Decal Solution containing HCl acid (Scientific Products) and processed for routine hematoxylin and eosin staining.

Sections for light microscopic histomorphometry were cut at 5-6 microns thickness and at 500 micron intervals throughout the length of the implant and/or control sites. Thus, each surgical site resulted in approximately 8-10 sections for data analysis.

All microscopic sections at each time interval were evaluated for rate of resportion of and host response to the PLA/PGA implant material, i.e., presence, intensity and character of any inflammatory infiltrate; presence of foreign body giant cells, macrophages and osteoclastic resorption of host bone. Further, each section was analyzed for surface area of regenerated bone

within the implant site as related to total surface area of the surgical defect. Thus, the percentage of new bone could be calculated and a mean value determined for all implant sites, thereby allowing for comparison of the difference between means for implant versus control sites and calculation of statistical significance using the Student T test. All histomorphometric measurements were accomplished with a commerically available computer software package (Sigma-Scan Measurement System, Jandel Scientific).

RESULTS

(Histology)

3 Days Post-Surgery

Control:

Slight to moderate inflammatory response consisting of neutrophils, macrophages and lymphocytes. Defect features a well formed fibrin clot. Evidence of capillary proliferation into defect from adjacent bone marrow spaces. Bone surfaces which form the proximal walls of the defect exhibit early osteoclastic mediated resorption. Two or three residual bony spicules remain in the defect in most sections.

Defects Filled With Implant Plug:

Same as control except that implant material fills most of defect and allows for very little clot formation.

Implant Material Positioned Over Superior and Inferior Surfaces of Defect:

Same as control - no apparent differences.

7 Days Post-Surgery:

Control:

Inflammatory cell infiltrate is rarely noted. Capillary and fibroblastic proliferation into defect appears very active and take their origin from both the adjacent marrow spaces and the periosteal and dura mater surfaces. Osteoclastic resorption of bony walls still evident.

Defects Filled With Implant Plug:

No evidence of inflammatory response or foreign body giant cell formation. Osteoclastic resorption of defect walls is generally less active than that of controls. Capillary infiltration of wound is apparent. Fibroblast migration into defect appears slightly less than control.

Implant Material Positioned Over Superior and Inferior Surfaces of Defect:

Inflammatory response similar to that of controls, i.e., rarely noted and consisting of a few lymphocytes. No evidence of foreign body giant cell formation. Osteoclastic resorption of proximal walls of defect. Capillary infiltration and fibroblast migration into defect is similar to that of controls.

14 Day Post-Surgery:

Control:

Periosteal and dura mater surfaces exhibit an intact fibrous connective tissue with fibers arranged parallel to the bony surface. Bony defect features trabecular bone formation. No evidence of osteoclastic activity.

Defects Filled With Implant Plug:

Periosteum and dura mater surfaces feature an intact fibrous connective tissue layer. No inflammation or evidence of foreign body giant cells. No evidence of bony repair or resorption of implant plug.

Implant Material Positioned Over Superior and Inferior Surfaces of Defect:

Defect features and intact periosteal and dura mater surfaces consisting of a well organized, dense layer of collagenous connective tissue. Evidence of bone formation via trabeculation.

21 Days Post-Surgery:

Control:

Defect almost completely filled with new trabecular bone.

Defects Filled With Implant Plug:

No evidence of foreign body giant cells or osteoclastic resorption of defect walls. General impression is that size and inert nature of plug has inhibited bony repair.

Implant Material Positioned Over Superior and Inferior Surfaces of Defect:

Defects filling with new trabecular bone but to a lesser degree than controls.

35 Days Post-Surgery:

Control:

Defects completely healed and filled with new bone.

Defects Filled With Implant Plug:

No apparent change from 21 Day samples except for very slight bone ingrowth at edges of defect.

Implant Material Positioned Over Superior and Inferior Surfaces of Defect:

Defects appear to be 80-90% healed.

RESULTS

(Computerized Histomorphometric Analysis)

A total of 40 microscopic sections from each experimental group at each time interval were examined by computerized histopmorphometry.

Mean Percent Surface Area of Regenerated Bone

	Controls (Group #1)	Defects Filled With Implant Plug (Group #2)	Implant Material Positioned Over Superior & Inferior Surfaces of Defect (Group #3)
3 Days	0	0	0
7 Days	5	0	0
14 Days	36	0	11
21 Days	94	1	72
35 Days	100	4	94

Statistical Significance ($p = > .05$):

14 Days: Group #1 vs. Group #2
Group #1 vs. Group #3
Group #3 vs. Group #2

21 Days: Group #1 vs. Group #2
Group #1 vs. Group #3
Group #3 vs. Group #2

35 Days: Group #1 vs. Group #2
Group #3 vs. Group #2

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